Genome Characterization of MT-2 Perennial and OK-906 Annual Wheat × Intermediate Wheatgrass Hybrids

T. A. Jones,* X-Y. Zhang, and R. R-C. Wang

ABSTRACT

Montana-2 (MT-2; × *Agrotriticum intermediodurum* Khizhnyak) is a variable perennial $F_{5:15}$ line derived from hybridization between durum wheat (*Triticum turgidum* L. var. *durum*, AABB) and *Thinopyrum intermedium* (Host) Barkw. & D.R. Dewey (StStEEEE). OK-906 is a uniform annual line derived from hybridization between hexaploid wheat (*Triticum aestivum* L., AABBDD) and an unknown *Thinopyrum* sp. followed by backcrossing to wheat and several generations of selfing and selection. We wished to characterize perennial (*Thinopyrum*) and annual (*Triticum*) chromosome content of MT-2 and OK-906 to determine how chromosome content corresponds to perenniality. When DNA of genomes ABD (*T. aestivum*) + E^b [*Thinopyrum bessarabicum* (Savul & Rayss) Löve] was used as the block and DNA of the genome St [*Pseudoroegneria stipifolia* (Czern. ex Nevski) A. Löve] was used as the probe, the average chromosome content among15 MT-2 lines was 26.2 wheat + 9.4 St + 18.8 E + 1.5 St/E translocation = 55.9 chromosomes (8*x*). Variation for genomic content was found within as well as among MT-2 lines, indicating that instability remains in the material. OK-906 exhibited about 40 wheat + 6 St + 8 E + 2 St/E translocation = 56 chromosomes (8*x*), but some aneuploidy was present. Its chromosome content is similar to 'Agrotana', another wheat × *Thinopyrum* annual. Perenniality in MT-2 may relate to its higher perennial chromosome dosage (about 30 of about 56 chromosomes) than OK-906 or Agrotana (16 of 56 chromosomes). Alternatively, specific genes or chromosome segments may confer perenniality. Development of stable breeding populations from MT-2 is improbable because of its variable chromosome constitution. However, individual lines could be useful for forage if they could be stabilized with improved seed yield.

Montana-2 perennial forage wheat was released as a germplasm (PI 505820) in 1986 by the Montana Agricultural Experiment Station (Schulz-Schaeffer and Haller, 1987). The seed weight of MT-2 lies between wheat and intermediate wheatgrass, but MT-2 is an unlikely perennial grain because of its inability to maintain yield through time (Schulz-Schaeffer and Friebe, 1992). While sterility of MT-2 is high, it is a potential perennial forage in areas where wheat is grazed as part of a forage—livestock production system. The original cross was made between durum wheat ($2n = 28$; **AABB**) as the female parent and intermediate wheatgrass (*Th. intermedium*) ($2n = 42$; $\mathbf{E^bE^bE^eE^eStSt}$ [Liu and Wang, 1993a] or alternatively, $\mathbf{EEE^{St}E^{St}StSt}$ [Chen et al., 1998]) as the male parent. We employ the standardized genome symbols designated by Wang et al. (1995).

The amphiploid was maintained at Beltsville, MD, and Bozeman, MT, from sometime prior to 1935 until 1986 when MT-2 was released as an $F_{5:15}$ line (Schulz-Schaeffer and Haller, 1987). Schulz-Schaeffer and Haller (1987) predicted that the chromosome number of the original amphiploid ($2n = 70$) would stabilize at the octoploid level ($2n = 56$) and hypothesized that, because of the *Triticum* cytoplasm, the 14 lost chromosomes would belong to the *Thinopyrum* parent.

The genomic formula of intermediate wheatgrass has been investigated by many researchers. On the basis of chromosome pairing in the normal hexaploid and a polyhaploid, Dewey (1962) showed this grass to be a segmental autoallohexaploid with two genomes more closely related to one another than either is to the third. Dvořák (1981) found the two similar genomes to be related to $\mathbf{E^e}$, the genome of *Th. elongatum* (Host) D.R. Dewey. On the basis of pairing data, Liu and Wang (1989) indicated that the genomic formula of *Th caespitosum* (C. Koch) R. R-C. Wang was $\mathbf{E^eE^eStSt}$. Because Dvořák (1981) had already shown that *Th caespitosum* ($4x$) and *Th. intermedium* ($6x$) have two homologous genomes, Liu and Wang (1989) concluded that the third genome of *Th. intermedium* was **St**. This conclusion is supported by karyotype analysis (Liu and Wang, 1993a). Chen et al. (1998) recently reported a new genomic formula for intermediate wheatgrass, JJJ^SJ^SSS (= $\mathbf{E^bE^bE^{St}E^{St}StSt}$), supported by genomic in situ hybridization (GISH) data. The new $\mathbf{E^{St}}$ genome exhibits modified **E** chromosomes distinguished by **St**-genomic sequences near the centromere. However, numbers of chromosomes corresponding to their **E** and $\mathbf{E^{St}}$ genomes varied among plants and were never 14.

Thinopyrum tetraploids may be $\mathbf{E^eE^eE^eE^e}$, $\mathbf{E^bE^bE^bE^b}$, or $\mathbf{E^eE^eE^bE^b}$ (Liu and Wang, 1993b); multiple hybridization events involving these species could have led to the evolution of intermediate wheatgrass. Therefore, the **E** genome make-up could vary among intermediate wheatgrass populations or the $\mathbf{E^e}$ and $\mathbf{E^b}$ genomes may have reconstituted in the hexaploid as a result of chromosome interchanges. Difficulty in preventing cross-hybridization of wheat chromosomes with **E** probes relative to **St** probes indicates that the **E** genomes are more closely related to the **A**, **B**, and **D** genomes of wheat than is the **St** genome (Zhang et al., 1996).

OK-906 is an octoploid ($2n = 56$) annual derived from a hexaploid wheat × *Thinopyrum* sp. amphiploid backcrossed to 'TAM-101' hexaploid wheat followed by 8 to 10 generations of selfing and selection (E.L. Smith, Oklahoma State University, 1996, personal communication). Our objectives were to survey chromosome variation within the genetically heterogeneous MT-2 and to determine whether this perennial differed genomically from OK-906.

MATERIALS AND METHODS

Genomic in situ hybridization was applied to two randomly selected progeny from each of 15 MT-2 plants grown in the

T.A. Jones and R. R-C. Wang, USDA–ARS, Forage & Range Research Lab., Utah State Univ., Logan, UT 84322-6300; X-Y. Zhang, Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China. Contribution of the Utah Agric. Exp. Stn. Paper no. 6091. Received 3 Aug. 1998. *Corresponding author (tomjones@cc.usu.edu).

Published in Crop Sci. 39:1041–1043 (1999).

field. These 15 parent plants were selected on an individual-plant basis for high seed yield. The GISH procedure was also applied to two plants of OK-906. Chromosome number was determined on 22 plants of OK-906.

Prepared slides were treated with RNase A (100 μg/mL in 2× SSC—1× SSC is 0.15 *M* NaCl plus 0.015 *M* sodium citrate) for 1 h at 37°C, denatured in 70% (v/v) formamide and 2× SSC for 2 min at 70°C, and dehydrated in a 70/80/95/100% (v/v) ethanol series at room temperature (Chen and Armstrong, 1994). The **St** genomic DNA probe (*Pseudoroegneria stipifolia*) was labelled with biotin-14-dATP via nick translation (BioNick Labelling System; Gibco BRL, Rockville, MD). The probe was mixed with 35× sheared *T. aestivum* (**AABBDD**) and *Th. bessarabicum* ($\mathbf{E^bE^b}$) DNA as a block (1:35 probe:block ratio), 35× salmon sperm DNA in 10 μL of 2× hybridization buffer (4× SSC, 200 m*M* phosphate, 2× Denhardt's solution [pH 6.5]), and 10 μL of 20% (w/v) dextran sulfate solution. Final probe concentration was 5 to 8 ng/μL.

The probe-block mixture solution was denatured at 70°C for 5 min, chilled on ice for 3 to 4 min, and preannealed at 37°C for 30 to 60 min. The probe solution was applied to a denatured slide (prewarmed at 40°C for about 1 h), topped with a coverslip, sealed with rubber cement, and incubated at 37°C overnight. Biotinylated goat anti-avidin D (5 μg/mL) and FITC-avidin D (10 μg/mL) were added to the slide. Finally, the slide was counterstained with 20 μL propidium iodide solution (0.5 μg/mL in glycerol containing anti-fading agent) for about 20 min (Chen and Armstrong, 1994; Zhang et al., 1996).

RESULTS AND DISCUSSION

Using DNA of the **St** genome as the probe and DNA of the **ABD** (*T. aestivum*) + $\mathbf{E^b}$ (*Th. bessarabicum*) genomes as the block, we were able to distinguish **St** chromosomes, which fluoresced yellow, from wheat chromosomes (**AB** in the case of MT-2 and **ABD** in the case of OK-906), which fluoresced red. Because **E** is closely related to **St**, but is intermediate between **St** and **ABD** (Zhang et al., 1996), **E** chromosomes fluoresced orange. However, regions near the centromere of **E** chromosomes often appeared yellow. Hybridization of the **St** probe in these highly repeated regions has been previously reported in *Th. ponticum* (Podp.) Liu & Wang (Zhang et al., 1996). This suggested to those authors the presence of a modified synthetic genome composed of chromosomes or chromosome segments from various genomes. They cited previous publications with data supporting the modified synthetic genome concept. Recently, Chen et al. (1998) interpreted such chromosomes (yellow centromeric region with orange arms under the stated GISH conditions) as a novel genome, $\mathbf{J^S}$, or $\mathbf{E^{St}}$ according to the nomenclature of Wang et al. (1995).

Table 1. Chromosome constitution of two individuals from each of 15 lines of MT-2 (× *Agrotriticum intermediodurum* Khizhnyak).

MT-2 line	A, B	St	E† ($E + E^{St}$)‡	St/E† ($St/E + St/E^{St}$)‡	Total
	no. of chromosomes				
1–4	26	10	20	2	58
	26	10	19	2	57
4–3	26	8	20	2	56
	28	6	18	2	54
9–2	28	10	18	0	56
	24	9	21	2	56
9–8	26	8	20	2	56
	26	8	20	2	56
10–2	24	12	20	0	56
	24	8	22	2	56
10–4	28	6	20	2	56
	28	8	20	0	56
10–5	28	8	14	6	56
	28	8	16	4	56
12–3	26	10	20	0	56
	26	10	18	2	56
13–2	28	8	18	2	56
	28	7	18	2	55
14–2	26	12	12	2	52
	28	12	14	2	56
15–7	28	14	14	0	56
	24	12	20	0	56
15–10	26	10	20	0	56
	26	10	20	0	56
16–6	26	8	21	2	57
	24	10	20	2	56
16–10	24	8	22	2	56
	24	8	22	2	56
17–3	24	12	20	0	56
	27	11	18	0	56
Mean	26.2	9.4	18.8	1.5	55.9
Range	(24–28)	(6–14)	(12–22)	(0–6)	(52–58)

† Interpretation by Liu and Wang (1993a).
‡ Interpretation by Chen et al. (1998).

Analysis of 30 MT-2 plants by GISH confirmed Schulz-Schaeffer and Haller's (1987) prediction of ploidy stabilization at the 8*x* level through loss of *Thinopyrum* chromosomes from the 10*x* amphiploid (Table 1). Mean chromosome number was 55.9. Numbers of *Thinopyrum* and wheat chromosomes averaged 29.7 and 26.2, respectively, indicating a mean loss from the amphiploid of 12.3 *Thinopyrum* and 1.8 wheat chromosomes. Nine of the 30 plants examined had a full complement of 28 wheat (**AABB**) chromosomes. Mean number of **E** chromosomes (18.8) was twice that of **St** chromosomes (9.4). This indicates that loss of **E** and **St** chromosomes was indiscriminate. Intermediate wheatgrass itself has two **E** genomes and one **St** genome.

Considerable genomic variation was found both among and within MT-2 lines. Because MT-2 was released as an F_5-derived line, variation among MT-2 lines was expected. Variation among lines is in agreement with results reported by Schulz-Schaeffer and Haller (1987). In 16 pollen mother cells of a single F_{14} plant, they found a range of 25 to 31 bivalents and 0 to 7 univalents, with total chromosome number ranging from 55 to 62. In our study, duplicate seedlings of the 15 lines gave identical karyotypes in only three cases (Lines 9–8, 15–10, and 16–10, Table 1).

Schulz-Schaeffer and Friebe (1992) reported 16 pairs of *Thinopyrum* chromsomes on the basis of C-banding results. However, sample size was not reported. We found that, while the number of *Thinopyrum* chromosomes was most often 32 (9 of 30 plants), the presence of 28 (8 plants) and 30 (7 plants) *Thinopyrum* chromosomes was also frequent (Table 1). The GISH technique also allowed us to detect **St/E** translocations, which could not be detected by C-banding. We also found variation within lines, indicating that chromosome content and number are not completely stable. On the basis of these results, additional generations of selfing and selection for high seed set will be required to derive stable lines from this material.

Genomes **A**, **B**, and **D** were confirmed in the 8*x* annual wheat × *Thinopyrum* hybrid, Agrotana (Conner et al., 1989; Xu et al., 1994). Initially, Agrotana was

thought to have 40 wheat + 14 *Thinopyrum* + 2 wheat-*Thinopyrum* translocation chromosomes = 56 (8*x*) (Xu et al., 1994), but further studies indicated chromosome content to be 40 wheat + 16 *Thinopyrum* = 56 (8*x*) (Chen et al., 1995). Likewise, we found no wheat–*Thinopyrum* translocations in MT-2, confirming the results of Schulz-Schaeffer and Friebe (1992).

Our analysis indicates the chromosome content of OK-906, a stable annual inbred line, is similar to Agrotana. Of 22 OK-906 plants counted, 10 were $2n = 55$, 10 were $2n = 56$, and 2 were $2n = 57$. We found 40 wheat + 6 **St** + 8 **E** (or $\mathbf{E^{St}}$) + 2 **St/E** (or $\mathbf{St/E^{St}}$) chromosomes = 56 (8*x*) in both OK-906 individuals analyzed with GISH. As in MT-2 and Agrotana, no wheat–*Thinopyrum* translocations were found in OK-906. Because **St** is present in OK-906, these data show that *Th. intermedium*, rather than *Th. ponticum*, is the perennial parent (Chen et al., 1998).

While we found no wheat-*Thinopyrum* translocations in MT-2, we found 5% of MT-2 chromosomes to be **St/E** (or $\mathbf{St/E^{St}}$) translocations (Table 1). These results indicate that **E** is more closely related to **St** than either is to **A** or **B**, as suggested by Zhang et al. (1996). Schulz-Schaeffer and Friebe (1992) reported a reciprocal translocation between chromosomes 1**B** and 2**B** in MT-2. Our technique was not designed to recognize translocations between wheat chromosomes.

Selection for improved seed set may stabilize MT-2 8*x* lines with **A**, **B**, and two synthetic *Thinopyrum* genomes consisting of a combination of **St** and **E** chromosomes. Individual lines may be extracted from MT-2, but Banks et al. (1993) argued that development of wheat × *Thinopyrum* amphiploids into a new crop is impractical. The content of the synthetic genomes would vary between plants, and the possibility of deriving multiple lines of the same genomic constitution needed for intercrossing is mathematically improbable. With advanced generations of inbreeding and selection, the possibility of deriving genomically similar lines increases. But as genetic variation between such lines decreases, the possibility of obtaining transgressive segregates from biparental crosses quickly declines.

Because perennial MT-2 has about one more perennial and one less annual genome than the annuals OK-906 and Agrotana, genome dosage could be the principal determinant of perenniality. When durum wheat is hybridized with intermediate wheatgrass, the wheat:*Thinopyrum* chromosome ratio in the initial amphiploid is 2:3, while in the amphiploid with hexaploid wheat the ratio is 1:1. If genome dosage is critical to perenniality, durum wheat may be preferred over hexaploid wheat as the *Triticum* parent when perennial forage is the objective. However, perenniality of MT-2 was strengthened by selection for survival after a series of harsh Montana winters (Schulz-Schaeffer and Haller, 1987).

Otrastayushchaya 38 and PGR-18752A are perennial 8*x* lines of amphiploid origin with 42 wheat + 14 *Th. intermedium* chromosomes (Banks et al., 1993). These lines have two more wheat chromosomes than OK-906, an annual. These workers reported the annual habit was expressed in 15 other 8*x* lines, also with 42 wheat + 14 *Th. intermedium* chromosome content. They believe that the perennial habit is conferred by only a few *Thinopyrum* chromosomes. Use of chromosome-specific fluorescent in situ hybridization probes can confirm whether individual MT-2 plants, Otrastayushchaya 38, and PGR-18752A carry common chromosomes that confer perenniality.

REFERENCES

Banks, P.M., S.J. Xu, R. R-C. Wang, and P.J. Larkin. 1993. Varying chromosome composition of 56-chromosome wheat × *Thinopyrum intermedium* partial amphiploids. Genome 36:207–215.

Chen, Qianfa, and K. Armstrong. 1994. Genomic *in situ* hybridization in *Avena sativa*. Genome 37:607–612.

Chen, Q., R.L. Conner, and A. Laroche. 1995. Identification of the parental chromosomes of the wheat-alien amphiploid *Agrotana* by genomic *in situ* hybridization. Genome 38:1163–1169.

Chen, Q., R.L. Conner, A. Laroche, and J.B. Thomas. 1998. Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic in situ hybridization. Genome 41:580–586.

Conner, R.L., E.D.P. Whelan, and M.D. MacDonald. 1989. Identification of sources of resistance to common root rot in wheat-alien amphiploid and chromosome substitution lines. Crop Sci. 29: 916–919.

Dewey, D.R. 1962. The genome structure of intermediate wheatgrass. J. Hered. 53:282–290.

Dvořák, J. 1981. Genome relationships among *Elytrigia* (*Agropyron*) *elongata*, *E. stipifolia*, "*E. elongata* 4*x*", *E. caespitosa*, *E. intermedia*, and "*E. elongata* 10*x*". Can. J. Genet. Cytol. 23:481–492.

Liu, Z-W., and R. R-C. Wang. 1989. Genome analysis of *Thinopyrum caespitosum*. Genome 32:141–145.

Liu, Z-W., and R. R-C. Wang. 1993a. Genome analysis of *Elytrigia caespitosa*, *Lophopyrum nodosum*, *Pseudoroegneria geniculata* ssp. *scythica*, and *Thinopyrum intermedium* (Triticeae: Gramineae). Genome 36:102–111.

Liu, Z-W., and R. R-C. Wang. 1993b. Genome constitutions of *Thinopyrum curvifolium*, *T. scirpeum*, *T. distichum*, and *T. junceum* (Triticeae: Gramineae). Genome 36:641–651.

Schulz-Schaeffer, J., and B. Friebe. 1992. Karyological characterization of a partial amphiploid, *Triticum turgidum* L. var. *durum* × *Agropyron intermedium* (Host) P.B. Euphytica 62:83–88.

Schulz-Schaeffer, J., and S.E. Haller. 1987. Registration of Montana-2 perennial × *Agrotriticum intermediodurum* Khizhnyak. Crop Sci. 27:822–823.

Wang, R. R-C., R. von Bothmer, J. Dvořák, G. Fedak, L. O'Donoughue, and K.C. Armstrong. 1995. Genome symbols in the Triticeae (Poaceae). p. 29–34. *In* R. R-C. Wang et al. (ed.) Proc. 2nd Int. Triticeae Symp., Logan, UT. 20–24 June 1994. Utah State University Publication Design and Production, Utah State University, Logan.

Xu, J., R.L. Conner, and A. Laroche. 1994. C-banding and fluorescence *in situ* hybridization studies of the wheat-alien hybrid 'Agrotana'. Genome 37:477–481.

Zhang, X-Y., Y-S. Dong, and R. R-C. Wang. 1996. Characterization of genomes and chromosomes in partial amphiploids of the hybrid *Triticum aestivum* × *Thinopyrum ponticum* by *in situ* hybridization, isozyme analysis, and RAPD. Genome 39:1062–1071.